

Expression and Association of SCD Gene Polymorphisms and Fatty Acid Compositions in Chicken Cross

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ABSTRACT

Stearoyl-CoA desaturase (SCD) is an integral membrane protein of endoplasmic reticulum (ER) that catalyzes the rate limiting step in the monounsaturated fatty acids from saturated fatty acids. Selection for fatty acids traits based on molecular marker assisted selection is needed to increase a value of chicken meat. This study was designed to analyze expression and associations of SCD gene polymorphisms with fatty acid traits in F2 kampung-broiler chicken cross. A total of 62 F2 kampung-broiler chicken cross (29 males and 33 females) were used in this study. Fatty acid traits were measured at 26 weeks of age. Samples were divided into two groups based on fatty acid traits (the highest and the lowest). Primers in exon 2 region were designed from the genomic chicken sequence. The SNP g.37284A>G was detected and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was then used to genotype. The expression of SCD gene was analyzed using quantitative real time PCR (qRT-PCR). The result showed that there were three genotypes (AA, AG, and GG) found in this study. The SCD|*AcI* polymorphism was significantly associated with palmitoleic acid (C16:1), fatty acids total and saturated fatty acid in 26 weeks old of F2 kampung-broiler chicken cross ($P < 0.05$). The SCD gene was expressed for polyunsaturated fatty acids in liver tissue in two groups of chickens. In conclusion, the SCD gene could be a candidate gene that affects fatty acids traits in F2 kampung-broiler chicken cross.

Keywords: kampung-broiler chicken cross, fatty acid, SCD gene, polymorphism, expression

ABSTRAK

Stearoyl-CoA desaturase (SCD) merupakan sebuah protein membran pada retikulum endoplasma (RE) yang mengkatalis tahap laju pembatasan asam lemak tak jenuh tunggal dari asam lemak jenuh. Program seleksi untuk asam lemak yang didasarkan dengan penanda molekuler dibutuhkan untuk meningkatkan nilai dari daging ayam. Penelitian ini didesain untuk menganalisis ekspresi gen SCD dan asosiasinya dengan sifat asam lemak pada ayam silangan kampung-broiler G2. Sebanyak 62 ekor ayam silangan kampung-broiler (29 jantan dan 33 betina) digunakan dalam penelitian ini. Sifat asam lemak diukur pada umur 26 minggu. Sampel dibagi kedalam dua grup berdasarkan sifat asam lemaknya (tertinggi dan terendah). Primer pada daerah exon 2 didesain berdasarkan runutan basa genom ayam. Sebuah SNP g.37284A>G dideteksi dan kemudian metode *polymerase chain reaction-restriction fragment length polymorphism* (PCR-RFLP) digunakan untuk menentukan genotipe. Ekspresi gen SCD dianalisis menggunakan kuantitatif real time PCR. Hasil menunjukkan bahwa terdapat tiga genotipe (AA, AG, dan GG) yang ditemukan pada penelitian ini. Keragaman SCD|*AcI* berasosiasi dengan asam lemak palmitoleat (C16:1), total asam lemak dan asam lemak jenuh pada ayam silangan kampung-broiler G2 ($P < 0.05$). Gen SCD juga terekspresi untuk sifat asam lemak tak jenuh ganda pada organ hati di kedua grup ayam. Dapat disimpulkan bahwa gene SCD dapat dijadikan kandidat gen yang mempengaruhi sifat asam lemak pada ayam silangan kampung-broiler G2.

Kata kunci: ayam silangan kampung-broiler, asam lemak, gen SCD, keragaman, ekspresi

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INTRODUCTION

Indonesian native chickens are important genetic resources, particularly for growth, meat and egg production. Commonly, Indonesian native chickens are raised using traditional production techniques but have a good adaptation to the tropical climate in Indonesia (Abubakar *et al.*, 2014). They are not considered as the main source of family income and just as a side-line activity. The productivity of Indonesian native chicken is still low (Sulandari *et al.*, 2007) but can be increased with better management and genetic improvement. In the other hand, Indonesian native chickens supply meat that has specific texture and taste. It contains lower fat and are preferred by consumers. As a result, native chicken meat is more expensive than broiler meat. There is a high potential for genetic improvement of indigenous chickens due to large genetic variation in production and meat quality traits. Crossbreeding program with broiler can be done to improve the genetic productivity of Indonesian native chicken.

Identifying candidate genes related to meat quality traits will provide an opportunity for genetic improvement in breeding programs, especially in fatty acids traits. These traits affect not only taste and texture in meat but also healthy status for the human. The study of molecular genetics and genomic technologies have led to the improvement of meat quality of farm animals (Gao *et al.*, 2007). An expression study and a polymorphic locus associated with fatty acids traits can be used as genetic markers. The genetic markers can be used for genetic improvement in the breeding program. (Ghanem *et al.*, 2016)

Stearoyl-CoA desaturase (SCD) is an intrinsic membrane protein that binds to the endoplasmic reticulum (ER) and catalyzes the rate limiting step in the monounsaturated fatty acids from saturated fatty acids (Heinemann & Ozols, 2003). There are 5 variants of SCD called SCD1, SCD2, SCD3, SCD4 and SCD5 in different organisms (Paton & Ntambi, 2009; Flowers & Ntambi, 2008; Castro *et al.*, 2011). The SCD gene is encoding an enzyme that catalyzes delta (Δ) 9 desaturation of SFA and MUFA (Ohsaki *et al.*, 2009). The SCD gene encoded delta (Δ) 9 desaturation to produce palmitoleic acid (C16:1) and oleic acid (C18:1), both of C16:1 and C18:1 fatty acid diversely expressed in the physiological process (Wu *et al.*, 2013; Alexander *et al.*, 2007).

Resnyk *et al.* (2013) reported that SCD gene was expressed in abdominal fat in fat and lean chicken. In the chicken, SCD gene expression is regulated by leptin, cerulenin, nutritional state, and gender in a tissue-specific manner (Dridi *et al.*, 2007). In another study, SCD gene expression was affected by age (Bohannon-Stewart *et al.*, 2014). In mammals, the expression of SCD gene was analyzed and associated with fatness and fatty acids (Maharani *et al.* 2013b; Oh *et al.*, 2013; Lim *et al.*, 2015)

The objectives of this study were to analyze an expression of SCD gene using RT-PCR, identify a polymorphism of SCD gene exon2 using PCR-RFLP and evaluate associations between a polymorphism of SCD gene and fatty acids compositions on F2 kampung-broiler chicken cross.

MATERIAL AND METHODS

Experimental Populations and Management

A total of 29 males and 33 females Indonesian kampung-broiler chicken cross at 26 weeks of age were used in the current study. The blood ratio of Indonesian kampung-broiler chicken cross (F2) was 25% kampung, 50% kampung-broiler and 25% broiler (strain Cobb). The samples were divided into two groups (the highest and the lowest) according fatty acids traits for gene expression study. All chickens had access to feed and water ad libitum. From hatch to 8 week of age, chickens received a starter feed (4,080 kcal of gross energy/kg and 19,03% of crude protein) and from 9 to 24 weeks of age, chickens were fed a grower diet (4,001 kcal of gross energy /kg and 17,42% of crude protein).

Phenotypic Measurements

Fatty acids traits were analyzed at 26 weeks of age. A part of leg chicken was used for fatty acids analysis. Fatty acids analysis was done according to Association of Official Analytical Chemists (AOAC, 2012). These measurements included fat content (FC), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and total fatty acids (TFA) .

RNA Isolation and qRT-PCR

The liver of chickens (n=3 per group) was extracted with JET RNA Purification Kit (Thermo Scientific, Lithuania, EU) and then stored at -20°C temperature. In this study, RNA was transcribed into complementary DNA (cDNA) using Transcriptor Synthesis First Strand cDNA Kit (Thermo Scientific, Lithuania, EU). RNA concentrations were measured with a NanoDrop spectrophotometer (NanoDrop Spectrophotometers 2000, Thermo Scientific, USA). PCR primers were designed in MEGA 5.0 and checked the profile using Primer Stat (SCD Forward: 5'-GGT GAC CAA GAA TGG GAA TG-'3, SCD reverse: 5'-CCA ATA ATG GCC CCT AGA TG-'3, GAPDH forward: 5'-GTG TTG TGG ACT TGA TGG TC-'3, GAPDH reverse: 5'-GAC AGA AGG GAA CAG AAC TG-'3). The length of mRNA product of SCD and GAPDH genes were 192 and 160 bp respectively. All RT-qPCR assays were conducted using SYBR Green Select Master Kit (Applied biosystem, USA). The reaction was done in 10 μ L containing 50 ng of total RNA and 0.4 μ M of each primer. Thermal cycles contained one cycle of pre-incubation at 95°C for 5 min, 35 cycles of amplification (95°C for 10 s and 60°C for 20 s). Data of qRT-PCR were analyzed using 2 $^{-\Delta\Delta CT}$ method (Schmittgen & Livak, 2008).

DNA Isolation and PCR Amplification

Blood samples were collected from *Vena axillaris*. Genomic DNA was isolated according to Sambrook *et al.*, (1989). The PCR primers of SCD gene exon 2 used in this study are based on a previously study (Maharani

et al., 2013). These primers (5' CCC CCA GAA AGA AAA AGT CC 3' and 5' CAA AAA TCC CAC CCA ACA AC 3') were designed to amplify a 468-bp fragment by primer stat according to the chicken genomic sequence in the GenBank database (accession number NC_006093). The PCR reaction conditions were 94°C for 5 min, 35 cycles of 94°C for 10 s, 60°C for 20 s, 72°C for 30 s, and an extension at 72°C for 5 min. The 25-μL reaction volume included 50 ng of DNA template, 1× reaction buffer, 5 pmol of each primer, 0.16 mM of deoxyribonucleotide triphosphate, 1.5 mM of MgCl₂, and 1 U of *Taq* polymerase.

Screening of the Population for Restriction Enzyme-Detectable SNP

A single nucleotide polymorphism (SNP) of the SCD gene was detected by digesting 7 μL of the 468 bp PCR product with 3 U of the *Acil* enzyme (Thermo Fisher Scientific Inc.) at 37°C overnight. The restriction digests were electrophoresed for 45 min at 100 V on a 2.0% agarose gel with ethidium bromide. Individual PCR-RFLP fragment sizes for the gene were determined by visualizing the band pattern under UV Transilluminator (AlphaImager® EP).

Statistical Analysis

Genotype and allele frequencies. The genotype and allele frequencies were analyzed using genotyping data based on the populations of kampung chicken (Nei & Kumar, 2000). Genotype frequency was calculated by the following formula:

$$X_{ii} = n_{ii} / N$$

Allele frequency was calculated by the following formula:

$$X_i = (2n_{ii} + \sum n_{ij}) / 2N$$

Description:

X_{ii} = i_{th} genotype frequency

X_i = i_{th} allele frequency

n_{ii} = Number of the sample of ii genotype

n_{ij} = Number of the sample of ij genotype

N = Total samples

Association study. The association between the polymorphism and body composition traits was analyzed using the GLM procedure (SAS Inst. Inc., Cary, NC). The model was fitted with the genotype (G; 3 levels; AA, AG, GG) as random effects, as follows:

$$Y = \mu + G + S + e,$$

Where Y is the dependent variable for trait measured in the population, μ is the overall population mean for traits, G is the genotype effects, S is sex effects and e is the random error. Significant differences between means of the different genotypes were calculated using the Duncan test. Significance was determined as $P < 0.05$, unless otherwise specified.

RESULTS

An Expression Study of SCD Gene

In this study, mRNA expression analyzed by qRT-PCR demonstrated that the SCD gene was expressed in liver tissue. There were five fatty acid traits analyzed for gene expression (Figure 1). The chickens were divided into two groups for each trait (the highest and the lowest level). According to relative expression between SCD and GAPDH gene (housekeeping gene), there were no different expression between the highest and lowest group on fat content, total fatty acid, saturated fatty acids and monounsaturated fatty acids. There was the only different expression on polysaturated fatty acids between the highest and lowest group in the kampung-broiler chicken cross.

A Polymorphism Study of SCD Gene

The 468 bp fragment of second exon of SCD gene was successfully amplified and shown in Figure 2. Three genotypes were detected and defined as AA, AG and GG. The 468 bp fragment was successfully digested with *Acil* restriction enzyme. The digested PCR products had fragment sizes of 236, 141 and 91 bp for the GG genotype, 327 bp and 91 bp for the AA genotype, and a combination of 327, 236, 141 and 91 bp for AG genotype. According to SCD|*Acil* locus polymorphism in F2 kampung-broiler chicken cross, the genotype frequency of AA, AG and GG were 0.306, 0.484, and 0.210 respectively. The allele frequency of A and G were 0.548 and 0.452 respectively.

An Association Study of SCD Gene Polymorphism and Fatty Acids Traits

The association of SCD gene polymorphism and fatty acid traits in 26 weeks old kampung-broiler chicken cross was summarized in the Table 1. The g.37284A>G SNP of SCD gene was generally significantly associated with fatty acids composition in 26 weeks old chicken. There were significant associations between the SCD polymorphism and fatty acids C16:1 (palmitoleic acids), total fatty acids, and saturated fatty acids in 26 weeks old chicken. No significant difference was observed in fat content, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA).

DISCUSSION

In expression study, the SCD gene was successfully expressed in liver tissue in F2 kampung-broiler chicken cross. According to relative expression, there were no the different expression on fat content, total fatty acid, saturated fatty acids and monounsaturated fatty acids. There was only the different expression on polysaturated fatty acids in liver tissue. This result indicated that SCD gene expression affected polysaturated fatty acids (PUFA) on final product of the fatty acid conversion in liver tissue of chicken. In another study, the expression profile of SCD gene in liver was different in response

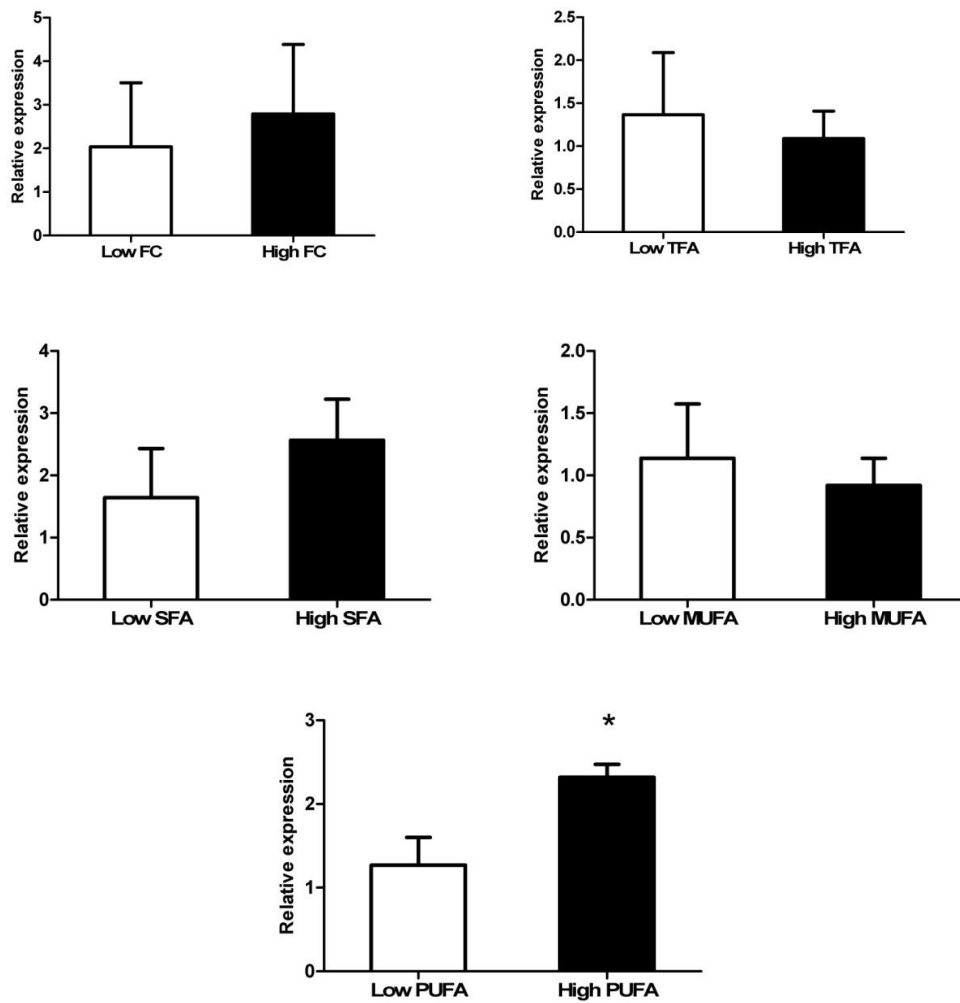


Figure 1. SCD mRNA Expression in liver tissue from group 1 and group 2 by qRT-PCR

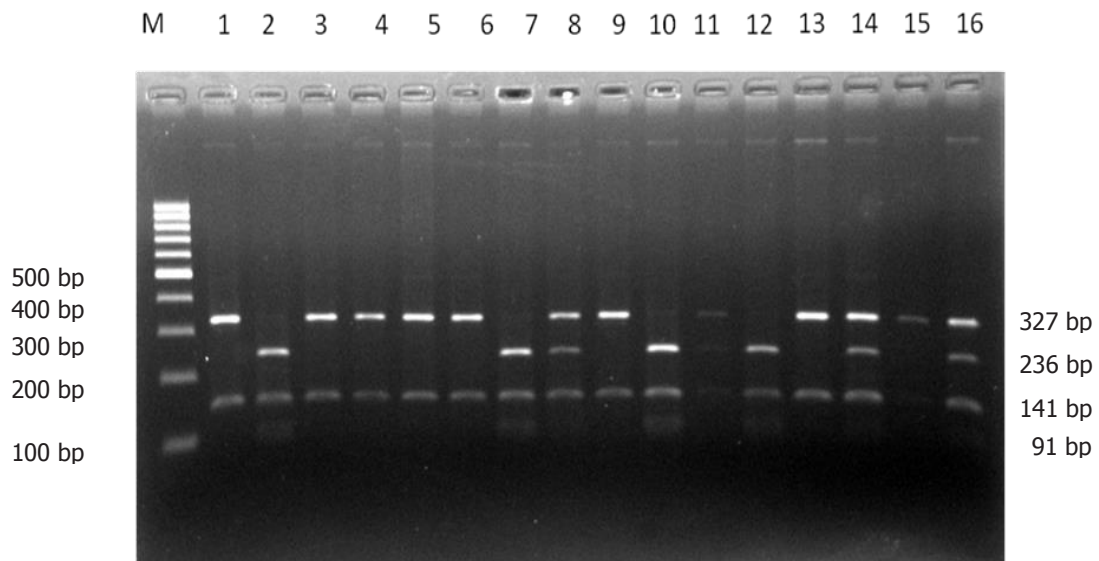


Figure 2. The PCR-RFLP pattern for SCD gene exon 2 region with *Acil* restriction enzyme. M= 100bp markers; 1,3-6,9,13,15= AA genotype; 2,7,10,12= GG genotype; 8,11,14,16= AG genotype.

Table 1. Fatty acids composition of Indonesian Kampung-broiler chicken cross at different genotypes (Means \pm SD)

Fatty acids composition ³	Genotype		
	AA(n=19)	AG(n=30)	GG(n=13)
Fat content	4.85 \pm 1.42	4.47 \pm 1.64	4.58 \pm 1.66
C12	0.05 \pm 0.01	0.04 \pm 0.01	0.05 \pm 0.02
C14	0.57 \pm 0.04	0.53 \pm 0.05	0.54 \pm 0.04
C14:1	0.07 \pm 0.03	0.08 \pm 0.04	0.08 \pm 0.02
C15	0.11 \pm 0.08	0.09 \pm 0.01	0.08 \pm 0.01
C16	20.56 \pm 1.35	19.06 \pm 1.47	19.70 \pm 1.78
C16:1	2.32 \pm 0.82 ^b	2.44 \pm 0.69 ^{ab}	2.69 \pm 0.80 ^a
C17	0.17 \pm 0.03	0.17 \pm 0.03	0.17 \pm 0.02
C18	5.63 \pm 0.74	5.06 \pm 0.70	5.17 \pm 0.64
C18:1n9t	0.16 \pm 0.03	0.14 \pm 0.03	0.14 \pm 0.03
C18:1n9c	31.78 \pm 1.86	31.05 \pm 2.01	31.23 \pm 2.16
C18:2n6c	20.78 \pm 2.03	20.77 \pm 1.61	20.16 \pm 1.79
C20	0.16 \pm 0.03	0.17 \pm 0.04	0.16 \pm 0.03
C18:3n6	0.10 \pm 0.02	0.10 \pm 0.02	0.09 \pm 0.01
C20:2	0.16 \pm 0.03	0.17 \pm 0.03	0.15 \pm 0.02
C22	0.05 \pm 0.02	0.06 \pm 0.03	0.06 \pm 0.01
C20:4n6	0.68 \pm 0.28	0.71 \pm 0.29	0.69 \pm 0.19
C22:6n3	0.06 \pm 0.02	0.07 \pm 0.03	0.07 \pm 0.03
Fatty acid total	83.44 \pm 3.68 ^a	80.74 \pm 4.25 ^b	81.28 \pm 5.17 ^b
Saturated fatty acid	21.66 \pm 1.37 ^a	20.11 \pm 1.48 ^b	20.76 \pm 1.81 ^b
MUFA ¹	34.34 \pm 2.50	33.71 \pm 2.49	34.15 \pm 2.75
PUFA ²	21.78 \pm 2.12	21.81 \pm 1.70	21.16 \pm 1.84

Note:

Means in the same row with different superscripts differ significantly ($P < 0.05$).

¹ MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids; ² Numbers shown in parentheses are the number of individuals with the specified genotype; ³ All of fatty acids compositions are in percent.

to fasting in chickens (Desert *et al.*, 2008). The identified SNP confirmed in exon might play important role in transcription process (Gunawan *et al.*, 2011). A polymorphism in the coding region directly affected the gene expression through changing the nucleotide sequence and structure of the gene and probably leading to change in mRNA synthesis, maturation, degradation, transportation, splicing or translation (Iida & Akashi, 2000).

In polymorphism study, SCD gene fragment were successfully amplified using PCR for all samples. There was a A/G SNP at base 37284 (accession number NC_006093). The PCR-RFLP method was used successfully for genotyping the SNP in exon 2 of the SCD gene. The genotype and allele frequencies were calculated in crossbred chicken (Table 2). There were three genotypes (AA, AG, and GG) and two alleles (A and G) found in this population. The AG genotype was the highest genotype frequency in crossbred chicken. The A allele

was more frequent than G allele in two groups of the kampung-broiler chicken cross. This was different with the results of Maharani *et al.*, (2013a) in the broiler. In the previous study, the genotype frequency of GG was the highest.

In the association study, there were associations between SCD|*Acil* locus polymorphism and fatty acid traits (palmitic acids, total fatty acids, and saturated fatty acid). The homozygous GG was significantly higher than the homozygous AA and heterozygous AG in palmitoleic acid (C16:1). The effect of this SNP indicated the increase palmitoleic acid in the homozygous GG. In fatty acids total and saturated fatty acids, the homozygous AA was significantly higher than the homozygous GG and heterozygous AG. This result indicated that homozygous AA could be a candidate marker related saturated fatty acid. In mammals, three SNPs of SCD gene in 3'UTR were associated with fat deposition and fatty

Table 2. Genotype and allele frequencies of SCD gene g.37284A>G SNP in kampung-broiler chicken cross (F2)

Population	N	Genotype Frequency			Allele Frequency	
		AA	AG	GG	A	G
26 week old in kampung-broiler chicken cross (F2)	62	0.306	0.484	0.21	0.548	0.452

acids compositions in cattle (Jiang *et al.* 2008). Maharani *et al.* (2013b) reported that the SNPs of SCD gene were associated with fatty acids compositions in pig.

In this study, the result was similar with Maharani *et al.* (2013a) for palmitoleic acids. Maharani *et al.* (2013a) reported that SCD \downarrow Acil polymorphism associated with palmitic acid (C16), palmitoleic acid (C16:1), and saturated fatty acids. In the previous study, The GG genotype was the highest on palmitoleic acid (C16:1) and AG genotype was the highest on palmitic acid (C16) and saturated fatty acids. Palmitoleic acid had a positive effect to reduce not only bad cholesterol (Nestel *et al.*, 1994) but also fat deposition in blood vessels and blood clot formation (Grundy, 1994). In many studies, the increasing of unsaturated fatty acids could improve flavor of meat and health benefits (Erkkilä *et al.*, 2008; Webb & O'Neill, 2008; Zhang *et al.*, 2008).

CONCLUSION

There was the different expression of SCD gene in liver tissue of crossbred chicken for polyunsaturated fatty acids (PUFA). There were three genotypes (AA, AG, and GG) found in this study. The SCD gene polymorphism exon 2 was significantly associated with palmitoleic acid (C16:1), total fatty acid and PUFA in 26 weeks old crossbred chicken. The SNP can be used as a potential marker to enhance the genetic improvement for the breeding program of chicken.

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